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Protective role of TD0014 against sodium valproate-induced reproductive toxicity in male wistar rats

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ABSTRACT

Sodium valproate (VPA), a common treatment of epilepsy and other diseases, is known to have severe toxic effects on testis both in experimental animals and in humans. This study was carried out to assess the protective activities of the TD0014 against sodium valproate (SVP)-induced reproductive toxicity in male rats. Animals were treated with TD0014 at the dose of 1.8 g/kg/day and 5.4 g/kg/day, and co-administered with SVP (500 mg/kg) for 7 weeks before all reproductive parameters were determined. The results showed all doses of TD0014 significantly protected the decrease testicular weight and testosterone level in SVP rats. TD0014 significantly improved the decrease sperm count and sperm motility in SVP treated rats. Moreover, testicular histology of TD0014 + SVP groups showed declining of testicular histopathologies as compared to SVP group. Therefore, it seems that TD0014 can prevent testicular and spermatozoal damage in male rats induced with SVP. The higher protective effect was seen with TD0014 at 5.4 g/kg dose.

Keywords: TD0014, sodium valproate, male rat

1. INTRODUCTION

Infertility is one of the major health problems in life, which affects 8–12% of couples worldwide. Of all infertility cases, approximately 40–50% is due to "male factor" infertility (McNamara, 2011). One of the male factor infertility is male hypogonadism, which is characterized by a deficiency in testosterone – a critical hormone for sexual, cognitive, and body function and development. Clinically low testosterone levels can lead to the absence of secondary sex characteristics, infertility, muscle wasting, and other abnormalities. In individuals who also present with clinical signs and symptoms, clinical guidelines recommend treatment with testosterone replacement therapy

(TRT) to improve quality of life,sense of well-being, sexual function, muscle strength and bone mineral density (Dohle et al., 2015). However, before prescribing TRT, one must be conscientious of its adverse effects. Data on the safety of TRT specific toour aging population is not currently available; however TRT has been linked to prostate cancer, benign prostatic hyperplasia, polycythemia and obstructive sleep apnea (Osterberg et al., 2014). Thus, there is a continuing need for the development of new effective therapies to treat patients with male hypogonadism. Following the current trends in medicine, many medicinal plants that considered a promising source for safe natural agents have been used in searching for alternative treatments to prevent and treat many health problems, including male hypogonadism.

TD0014 is a preparation of herbal medicines which comprises thirty-three medicinal plants. The composition of TD0014 has several medicinal herbs that have been studied and used since ancient times in traditional folk medicine as an aphrodisiac. However, no studies have provided reliable shreds of evidence of their effects on reproductive functions when combining them in TD0014. Therefore, the purposes of this study were to evaluate the protective role of TD0014 against sodium valproate-induced reproductive toxicity in male rats.

2. MATERIAL AND METHOD

This study was prospectively carried out at National Hospital of Traditional Medicine, from September 2020 toApril 2021

Polyherbal formula TD0014 preparation

TD0014 was manufactured as hard pills according to the quality standard of Sunstar Joint Stock Company, Vietnam. The main ingredients of the herbal formulawereobtained from 32 herbs and 1 animal resource (per 7.5g of serving): *Tribulus terrestris* (4 g), *Chrysanthemum sinense* (1.83 g), *Prunus persica* (1.14 g), *Vigna cylindrica* (1.14 g), *Eurycoma longifolia* (0.69 g), *Sophora japonica* (0.57g), *Dioscorea persimilis* (0.43 g), *Dioscorea tokoro* (0.4 g), *Polygonum multiflorum* (0.4 g), *Citrus deliciosa* (0.34 g), *Polyscias fruticosa* (0.34 g), *Tinospora sinensis* (0.29 g), *Chaenomeles lagenaria* (0.29 g), *Passiflora foetida* (0.29 g), *Zizyphus sativa* (0.29 g), *Rehmannia glutinosa* (0.23 g), *Angelica sinensis* (0.23 g), *Alisma plantago-aquatica* L. var. orientalis Samuelsson (0.23 g), *Achyranthes bidentata* (0.23 g), *Schizandra chinensis* (0.23 g), *Morinda offcinalis* (0.23 g), *Rosa laevigata* (0.23 g), *Allium sativum* (0.2 g), *Lycium sinense* (0.17 g), *Glycyrrhiza uralensis* (0.14 g), *Panax ginseng* (0.11 g), *Ligusticum wallichii* (0.11 g), *Cistanche tubulosa* (0.11 g), *Atractylodes macrocephala* (0.11 g), *Radix Codonopsis* (0.11 g), *Cuscuta sinensis* (0.11 g), *Psoralea corylifolia* (0.06 g), *Cornu Cervi parvum* (7.2 mg). The herbal mixture extracts satisfied the herb, heavy metals, general bacteria, fungi, and specific pathogens criteria, as determined by a confirmation test for each. The experimental animals drank the test drug mixed with pure water.

Animals

Mature male rats of *Wistar* strain weighing 180-220 g b.wt each and 12-14 week old, were used in our study. The rats were kept under controlled hygienic conditions in metal cages and fed on basal diet for one week before starting the experiment for acclimatization. Water was provided *ad libitum*. The experimental protocol was approved by the ethics committee of Hanoi Medical University, Vietnam.

Experimental design

The animals were randomly divided into the following four groups (n = 10):

Group I: Negative control –The male rats received orally distilled water (10 ml/kg b.wt.) for 7 weeks

Group II: Positive control - The animals received SVP in a dose of 500 mg/kg b.wt./day for 7 weeks

Group III: TD0014 at the dose of 1.8g/kg was orally givenalong with SVP in a dose of 500 mg/kg b.wt./day for 7 weeks

Group IV: TD0014 at the dose of 5.4 g/kg was orally administeredalong with SVP in a dose of 500 mg/kg b.wt./day for 7 weeks.

At the end of the experiment, all rats were weighed and killed by exsanguination. Blood samples from the carotid artery were collected and kept standing for 15 min to clot then centrifuged at 10,000 rpm for 10 min to separate the serum which kept frozen at −70°C. The serum was used for estimation of testosterone levels. Both testes of each rat were exposed by a longitudinal incision in the skin of scrotum. Semen samples were collected from cauda epididymis by cutting the tail of epididymis and squeezed it intoa clean watch glass. The semen samples were used for semen analysis. The testes and accessory sexual organs were dissected out and weighed, and relative weights of sexual organs were calculated. The right testes were preserved in 10% neutral formalin solution till processed for histological examination.

Statistical Analysis

Data were presented as means ± standard deviation. Difference between means in the experimental groups were tested for significance using Student's t-testand $p < 0.05$ were considered to be significant.

3. RESULTS

Oral administration of SVP to male rats in a dose of 500 mg/kg during 7 weeks of the experiment induced significant ($p < 0.05$) decreases in weights of testes and accessory sexual organs (glans penis, epididymis, seminal vesicles, ventral prostate, Cowper's glands and levator ani/bulbocavernosus muscles [LABC]) when compared to the normal control group. A daily oral administration of TD0014 for 7 consecutive weeks was followed by a significant increase in relative weights of the testes and some accessory organs when compared to SVP-intoxicated control group. TD0014 at the dose of 5.4 g/kg caused a significant increase in the weights of glans penis ($p < 0.01$), seminal vesicles ($p < 0.05$), epididymis ($p < 0.001$), and Cowper's glands ($p < 0.05$) of rats, while TD0014 at the dose of 1.8 g/kg caused only a significant increase in the weight of glans penis ($p < 0.05$) and epididymis ($p < 0.01$) (Table 1).

Table 1 Effect of TD0014 on weights of sexual organs in SVP-intoxicated rats

Weight (mg/100g b.wt)	Negative control	Positive control	TD0014 1.8 g/kg	TD0014 5.4 g/kg
Testes	1268,16 ± 105,48	603,26 ± 150,24***	1019,57 ± 273,42++	802,68 ± 158,30+
Glans penis	51,29 ± 9,93	39,25 ± 4,33*	50,62 ± 12,23+	54,43 ± 9,63++
Seminal vesicles	110,52 ± 27,59	65,45 ± 18,64**	70,27 ± 6,73	92,22 ± 24,69+
Epididymis	371,06 ± 39,86	175,40 ± 33,83***	268,41 ± 63,37++	281,92 ± 57,25+++
Ventral prostate	69,42 ± 19,67	32,61 ± 8,73***	33,60 ± 7,89	40,59 ± 7,65
Cowper's glands	17,17 ± 1,53	14,62 ± 2,13*	16,59 ± 3,93	18,02 ± 2,66+
LABC	252,18 ± 62,96	162,71 ± 36,97**	167,18 ± 33,68	190,51 ± 40,73

*p < 0.05, **p < 0.01, ***p < 0.001 as compared to negative control. +p < 0.01, ++p < 0.01, +++p < 0.001 as compared to positive control. LABC: levator ani/bulbocavernosus muscles

Oral administration of SVP (500 mg/kg) to rats during 7 weeks of the experimental period significantly decreased serum testosterone ($p < 0.001$) when compared with the normal control group. Co-administration of TD0014 with SVP significantly increased serum testosterone ($p < 0.001$) when compared with the intoxicated control group, in a dose-dependent fashion (Table 2).

Table 2 Effect of TD0014 on serum testosterone in SVP-intoxicated rats

Groups	Testosterone(nmol/L)
Group I: Negative control	4,17 ± 1,13
Group II: Positive control (SVP)	1,35 ± 0,44***
Group III: SVP + TD0014 (1.8 g/kg)	4,30 ± 1,10+++
Group IV: SVP + TD0014 (5.4 g/kg)	5,64 ± 1,03+++Δ

****p < 0.001 as compared to negative control; +++p < 0.001 as compared to positive control; Δp < 0.05 as compared to group III*

Table 3 Influence of TD0014 on the size of seminiferous tubule in SVP-intoxicated rats

Groups	The size of seminiferous tubule (pixell)
Group I: Negative control	452,74 ± 55,12
Group II: Positive control (SVP)	326,09 ± 38,81***
Group III: SVP + TD0014 (1.8 g/kg)	371,97 ± 25,62+
Group IV: SVP + TD0014 (5.4 g/kg)	462,20 ± 58,25+++ΔΔ

****p < 0.001 as compared to negative control; +p < 0.05, +++p < 0.001 as compared to positive control; ΔΔp < 0.01 as compared to group III*

Data shown in Table 3 revealed that intoxication of rats by SVP induced significant decreases in the size of seminiferous tubule compared with negative control rats. TD0014 with all tested doses significantly increased the size of seminiferous tubule, in a dose dependent fashion, when compared with positive intoxicated rats.

Table 4 Influence of TD0014 on sperm count, viability, and abnormality in SVP-intoxicated rats

Groups	Sperm characters		
	Count (10^6/mL)	Viability (%)	Abnormality (%)
Negative control	144.65 ± 17.34	93.63 ± 2.33	39.13 ± 4.91
Positive control	$3.83 \pm 1.17^{***}$	$59.83 \pm 12.73^{***}$	$70.83 \pm 1.17^{***}$
TD0014 1.8 g/kg	$18.33 \pm 5.85^{+++}$	51.67 ± 15.11	62.17 ± 11.07
TD0014 5.4 g/kg	$106.20 \pm 33.13^{+++\Delta\Delta\Delta}$	$88.70 \pm 6.18^{+++\Delta\Delta\Delta}$	$55.50 \pm 10.91^{++}$

$^{***}p < 0.001$ as compared to negative control; $^{++}p < 0.01$, $^{+++}p < 0.001$ as compared to positive control; $^{\Delta\Delta\Delta}p < 0.001$ as compared to group III

SVP when given orally to male rats (500 mg/kg) during 7 weeks of the experiment induced significant decreases in sperm viability and count and increased percentages of sperm abnormalities when compared with the normal control group. Treatment with TD0014 significantly increased sperm count, in a dose dependent manner, while the percentage of sperm viability and abnormality were only improved at the dose of 5.4 g/kg TD0014, as recorded in Table 4.

Table 5 Effect of TD0014 on sperm motility in SVP-intoxicated rats

Groups	Motility index (%)			
	Rapid motility	Slow motility	*In situ* motility	Immotile
Negative control	30.25 ± 8.81	12.25 ± 4.20	8.38 ± 2.13	49.13 ± 7.08
Positive control	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	$100.00 \pm 0.00^{***}$
TD0014 1.8 g/kg	0.00 ± 0.00	0.00 ± 0.00	11.17 ± 3.49	$88.83 \pm 3.49^{+++}$
TD0014 5.4 g/kg	22.80 ± 7.57	6.70 ± 2.16	$6.70 \pm 1.83^{\Delta\Delta}$	$63.80 \pm 9.44^{+++\Delta\Delta\Delta}$

$^{***}p < 0.001$ as compared to negative control; $^{+++}p < 0.001$ as compared to positive control; $^{\Delta\Delta\Delta}p < 0.001$ as compared to group III

Motility index were classified as immotile, *in situ* (sperm flagella beating without midpiece movement) and progressive movement (rapid and slow motility). Observing in Table 5, the percentage of immotile sperm was 100% in SVP-intoxicated rats. The semen of male rats received TD0014 at the dose of 1.8 g/kg had motile sperms, however, the spermatozoa have non-progressive motility, i.e it moved only *in situ*. The male rats exposed tomultiple oral dose of TD0014 at 5.4 g/kg/day were significantly improved the speed of movement of spermatozoa with the presence of rapid and slow progressive sperm. The preventive effects of TD0014 on testicular damage were evaluated by observing histopathological structures (Figure 1).

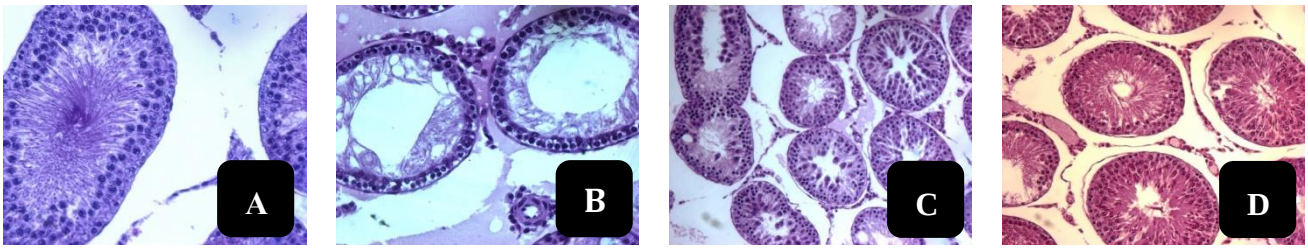

Figure 1 Photomicrographs of testes of: (A) Normal control rat showing normal histological structure of seminiferous tubules filled with mature sperms. Hematoxylen and eosin (H and E × 400), (B) intoxicated rat by sodium valproate (SVP) showing atrophied seminiferous tubules and edema with absence of sperms (H and E × 400), (C) Rat treated by the low dose (1.8 g/kg) of TD0014 showing partial improvement of the germinal epithelium of seminiferous tubules, but their lumen still wide and not full of sperm cell lineage (H and E × 400), (D) TD0014-treated rats showing seminiferous tubules with a narrow lumen filled by sperm cell lineage (H and E × 400).

4. DISCUSSION

Sodium valproate (SVP) is an antiepileptic drug commonly used in the treatments of epileptic seizures including panic attack, anorexia nervosa, anxiety disorder, posttraumatic stress disorder, migraine, psychiatric conditions, and bipolar disorders (McNamara, 2011). However, SVP has been reported to exert side effects on reproductive systems in both men and experimental animals. In the epileptic men treated with SVP, their testosterone levels and semen qualities have been significantly decreased (Isojärvi, 2008; Aldemir, 2009; Najafi et al., 2012) involving infertility. In animals, SVP significantly decreases FSH, LH, and testosterone hormones with testicular damages (Bairy et al., 2010; Nishimura et al., 2000). The purposes of this study were to evaluate the protective activities of TD0014 against testicular toxicity induced by SVP in rats. Data from the positive control group also demonstrated similar results to those previously reported about SVP toxicity on the structure and functions of sexual organs (Bairy et al., 2010; Nishimura et al., 2000): decreased weights of the testes and accessory sexual organs, low serum testosterone level, low semen quantity and quality, as well as incidence of testicular edema and necrosis with markedly atrophied seminiferous tubules.

In this study, the oral treatment with TD0014 at two dosage levels in SVP-intoxicated rats produced a protective effect against testicular toxicity. This protective effect characterized by increased weights of testes and some accessory sexual organs, improved semen quality and quantity, increased serum testosterone levels as well as partial amelioration of testicular histopathological lesions seen. This protective effect seemed to be more potent with the dose 5.4 g/kg than with the dose 1.8 g/kg. The positive effects of TD0014 on the testicular structure were shown in Table 1, Table 3 and Figure 1. TD0014 at both doses increased significantly the testis weight of SVP-intoxicated rats.

In the histopathological examination of the testis, this herbal remedy ameliorated partially the testicular histopathological lesions seen. The greater protective effect being observed for the group treated with TD0014 at 5.4 g/kg. The testes have two functions – to produce sperm and to produce hormones, particularly testosterone. The analysis of semen samples which were collected from cauda epididymis was used for evaluating the sperm production of testicles. Effects of TD0014 on sperm quantity and quality were recorded in Table 4 and Table 5. A dose-dependent increase in the sperm count was observed in the group treated with different doses of TD0014 (1.8 and 5.4 g/kg) compared to the toxic group. Meanwhile, the sperm quality index were only improved at the dose of 5.4 g/kg TD0014, with an increase in the sperm viability and motility, and a decrease in the sperm abnormalities. These results were consistent with a dose-dependent increase in the serum testosterone was shown in TD0014 groups. The sex accessory organsare known to be sensitive to testosterone level in serum. TD0014 elevated the level of testosterone so it tended to increase the weights of androgen-dependent accessory sex organs. There was a positive correlation between the increase in serum testosterone levelof each tested dose of TD0014 with the number of sex accessory organs increased significantly weight compared toposivite control group: TD0014 at the dose of 1.8 g/kg caused a significant increase in the weights of glans penis and epidydimis; TD0014 at the dose 5.4 g/kg, in addition to glans penis and epidydimis, also significantly increased the weights of seminal vesicles and Cowper's glands (Table 1).

From the above results, it can be seen that TD0014 expressed the protective effect of TD0014 against SVP-induced reproductive toxicity, which seems to be more potent with the dose 5.4 g/kg than with the dose 1.8 g/kg. The underlying mechanisms of the effects of SVP on reproductive functions have yet to be determined. One suggested mechanism is an increase in estradiol and testosterone antecedents following SVP use. This increase, decreases the release of pituitary hormones via the negative feedback mechanism. Decreased release of pituitary hormones may manifest as reproductive dysfunction (Aldemir, 2009). Addition, the most likely mode of action for SVP is potentiation of the inhibitory action of gammaamino-butyric acid (GABA) through an action on the further synthesis or further metabolism of GABA. It is also suggested that GABAergic neurotransmission may affect gonadotropin release (Isojärvi, 2008). SVP may also induce free radical formation and lipid peroxidation, which are chemical mechanisms capable of disrupting the structure and function of testis (Cárdenas-Rodríguez et al., 2013).

The mechanism involved in the protective effect of TD0014 against sodium valproate induced reproductive toxicity is unknown. However, based on the results of the individual studies of herbal components of TD0014, it is possible to partly explain the effectiveness of the herbal remedy on this research model. Many medicinal plants used in traditional medicine as the aphrodisiac agents, such as *Tribulus terrestris*, *Eurycoma longifolia* Jack, *Cistanches Herba*, *Morindaofficinalis*, *Lycium sinense*, *Cuscuta chinensis*, *Panax ginseng*, and *Psoralea corylifolia*, exhibited simultaneous the ability to raise the concentration of sex hormones (testosterone, LH, FSH) and the anti-oxidant activities on many experimental and clinical studies. Many other medicinal herbs in the herbal formula TD0014, although no studies have shown the effectiveness of these plants in improving reproductive functions, but have been found anti-oxidant properties, thatprevent damage to reproductive organs involving the formation of free radicals caused by SVP.

Tribulus terrestris (TT) (family Zygophyllaceae) has been used since ancient times in traditional folk medicine as an aphrodisiac and to treat urinary tract infections, inflammation, and other ailments.

The aphrodisiac properties of *TT* was explored in several studies, in which, administration of *TT* to humans and animals improves libidoand spermatogenesis (Chhatre et al., 2014). The two main components of the saponin fraction from *TT*, namely protodioscin and protogracillin, are responsible for the observed biological aphrodisiac activity (Adaikan et al., 2001). It is suggested that protodioscin works by increasing the levels of testosterone, leutinizing hormone (Koumanov et al., 1982), dehydroepiandrosterone (Adimoelja et al., 1997), dihydrotestosterone and dehydroepiandrosterone sulphate (Gauthaman et al., 2000), as well as increasing the conversion of testosterone into the potent dehydrotestosterone, which stimulates not only increase in the sex drive but also the production of red cells from bone marrow along with muscular developments contributing to improvement of blood circulation and the oxygen transport systems, leading tooptimal health (Chhatre et al., 2014). The anti-oxidant activity of *TT* was attributed to the presence of 4,5-di-p-coumaroylquinic acid that isolated from *TT* fruits and proved to exhibit a potent anti-oxidant effect (Hammoda et al., 2013).

Eurycoma longifolia Jack (*EL*) or commercially known as Cay ba binh in Vietnam (Tambi, 2002) is a famous medicinal plant in the family of Simaroubaceae. *EL* is well known for treating disease and enhancing health, particularly sexual health among men. Results published by Pramoto (2017) indicated that administration of the extract of *EL* has increased the activity of producer cells of LH to synthesize LH hormone in the anterior pituitary; thereby acting on Leydig cells to stimulate testosterone production, improving spermatogenesis of the testis. Eurycomanone, the highest concentrated quassinoid in the root extract of *EL*, enhanced testosterone steroidogenesis at the Leydig cells by inhibiting aromatase conversion of testosterone tooestrogen, and at a high concentration may also involve phosphodiesterase inhibition (Low et al., 2013). *EL* has been well documented to exert antioxidative properties due to its high concentrations of superoxide dismutase (SOD) (Pramoto, 2016; Tambi, 2005).

According to traditional medicine, *Cistanches Herba* also commonly used in the treatment of male hypogonadism. *Cistanche Herba* extract increased rat sex hormone levels by induction of testicular steroidgenic enzymes including CYP11A1, 3β-HSD, 17β-HSD and CYP17A1 (Wang et al., 2016; Jiang et al., 2016). Recent studies demonstrated the anti-oxidant activity of *Cistanches Herba*, particularly in the clearing of all types of free radicals *in vivo* and *in vitro*, improvement in the activity of anti-oxidant enzymes in the body such as SOD, CAT and GPX, and inhibition of the formation of lipid peroxide, MDA, and brown fat. The phenylethanoid glycosides from *Cistanches Herba* are considered the effective ingredients for anti-oxidative activities (Gu et al., 2016). In addition, the stimulating effect on the production and excretion of sex hormones and antioxidant properties of *Morindaofficinalis*, *Lycium sinense*, *Cuscuta chinensis*, *Panax ginseng*, and *Psoralea corylifolia* has shown in many different studies.

5. CONCLUSION

TD0014 is a preparation of herbal medicines which comprises thirty-three medicinal plants.TD0014 can prevent testicular and spermatozoal damage in male rats induced with SVP. The higher protective effect was observed with TD0014 at 5.4 g/kg dose.

Author contribution

All authors have contributed equally to this work. All authors have read and approved the final manuscript and agreed to publish this manuscript.

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Conflict of Interest

The authors declare that there is no conflicts of interests.

Ethical approval

The study was approved by the Medical Ethics Committee of National Hospital of Traditional Medicine (ethical approval code: 33/IBR-NHTM).

Data and materials availability

All data associated with this study are present in the paper.

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